

3C
Pesticide Protection
Franklin Research Center

612021-4
05006779
JOURNAL PROCEEDING: Environmental Science and
Technology

VOLUME: 12

ISSUE: 7

PAGES: 832-838

YEAR: 1978

AUTHOR: Lee, R.F.

ARTICLE TITLE: Fate of poly cyclic aromatic
hydro carbons in controlled
ecosystem enclosures

LANGUAGE: Eng
DATE: 06/21/79
SENT TO: 00001

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Fate of Polycyclic Aromatic Hydrocarbons in Controlled Ecosystem Enclosures 126 x 1 7

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■ Prudhoe crude oil enriched with a number of polycyclic aromatic hydrocarbons was added as a dispersion to a controlled ecosystem enclosure suspended in Saanich Inlet, Canada. Concentrations of various aromatics were determined in water, zooplankton, oysters, and bottom sediments. Initial water concentrations of the lower weight aromatics, naphthalenes and anthracene, were 10–20 µg/L, whereas the initial concentrations of benzo(a)pyrene, benz(a)anthracene, and fluoranthene ranged from 1 to 6 µg/L. These concentrations decreased at an exponential rate due to evaporation, photochemical oxidation, microbial degradation, and sedimentation. Only naphthalenes were significantly degraded by microbes with removal by this process of up to 5% per day from the water. Sedimentation and photochemical oxidation were responsible for the decrease in concentrations of the higher weight aromatics.

This paper summarizes an experimental study of the fate of a number of polycyclic aromatic hydrocarbons of increasing molecular weight in a controlled ecosystem enclosure. The hydrocarbons included naphthalene, methyl-naphthalenes, dimethyl-naphthalenes, anthracene, fluoranthene, benz(a)-anthracene, and benzo(a)pyrene. Prudhoe crude oil was enriched with these aromatics and added as a dispersion to a quarter-scale enclosure (ca. 2 m diameter and 15 m deep—60,000 L). After oil addition, water, sediment, zooplankton, and oysters in the enclosures were analyzed for the various hydrocarbons. A second experiment involved adding radiolabeled benzo(a)pyrene to an enclosure to allow quantification of hydrocarbon products.

The changes in the concentration of the different hydrocarbons in water are a reflection of the action of evaporation, biological degradation, photochemical oxidation, and adsorption to living or dead particles with subsequent sedimentation. Using the results of our experiments we have attempted to evaluate the importance of these processes as it affects different types of aromatic hydrocarbons.

Experimental

Polyethylene enclosures (ca. 2 m diameter and 15 m deep) were filled with 60,000 L of water from Saanich Inlet which is located in western Canada. Naphthalene (1 g), 1-methylnaphthalene (1 g), 2,3-dimethylnaphthalene (1 g), anthracene (1 g), fluoranthene (0.5 g), benz(a)anthracene (0.5 g), and benzo(a)pyrene (0.2 g), all from Aldrich Chemical Co., were dissolved in 100 g of Prudhoe crude oil (kindly donated by W. Cretnéy from Environment Canada, British Columbia). Prudhoe crude oil appears similar to other crudes, e.g., Kuwait and South Louisiana; therefore, the amounts of naphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, fluoranthene, benz(a)anthracene and benzo(a)pyrene contributed by the 100 g of crude oil would be 80, 300, 10, 0.2, and 0.1 mg, respectively (1–3). A dispersion of this oil with its spiked compounds was formed by mixing the oil with 1 L of acetone, 0.5 L of ethanol, and 40 L of seawater. This dispersion was

stirred for 24 h and then pumped through a diffusion ring throughout the top 10 m of the water column. One liter of acetone and 0.5 L of ethanol were pumped into a control enclosure. In a third enclosure 10 mCi of (³H) benzo(a)pyrene (20 mCi/mM—Amersham) and 72 mg of benzo(a)pyrene (Aldrich Chemical Co.) were dissolved in 6 L of acetone, then mixed with 20 L of seawater, and the dispersion pumped through the diffusion ring into the top 10 m of the enclosure.

Water samples were collected by a pump from three depths with a peristaltic pump through a flexible tube. Some water samples were also collected with a Niskin water sampler. Hydrocarbon concentrations were the same with both collecting methods, suggesting that the tubing was not adsorbing significant amounts of the hydrocarbons. Samples of bottom sediment were collected by pumping through flexible plastic pipes permanently connected to the bottom of the enclosure. Zooplankton samples were collected with a 50-cm-diameter, 200-µm mesh net.

For bioaccumulation studies a cage with oysters (*Crassostrea virginica*) was suspended at 7 m in the oil-treated enclosure. At various time intervals, oysters were removed for analysis or depuration work. For depuration studies oysters were removed from the polluted enclosures and placed in a second cage suspended in outside "clean" water.

For microbial degradation studies, water was collected from 3 and 7 m in the oil treated and control enclosures. Radiolabeled hydrocarbons (¹⁴C) dissolved in 2 µL of acetone were added to 100 mL water samples in 250-mL flasks capped with silicone stoppers. After incubation for various time intervals at the in situ temperature (12 °C) in the dark, the respired ¹⁴CO₂ released after the addition of acid was collected on filters soaked with phenylamine and counted. A more detailed description of the method is given by Hodson et al. (4). To determine if any chemical degradation to CO₂ occurred, 5 mg of mercuric chloride was added to some samples. Four samples were run for each time interval. The radioactive hydrocarbons used with 2-methylnaphthalene-8-¹⁴C (7.98 mCi/mM—California Bionuclear Corp.), ¹⁴C-1-naphthalene (3.67 mCi/mM—Amersham), anthracene-9,10-¹⁴C (33 mCi/mM—California Bionuclear Corp.), ¹⁴C-12-benz(a)anthracene (48 mCi/mM—Amersham), benzo(a)pyrene-3,6-¹⁴C (21 mCi/mM—Amersham).

For adsorption studies the radiolabeled hydrocarbons were added to 100-mL water samples. Concentrations of naphthalenes, anthracene, benz(a)anthracene, and benzo(a)pyrene were 25, 25, 10, and 3 µg/L, respectively. After incubation for 3 h at the in situ temperature, the water was filtered onto a 0.4-µ filter (Nucleopore Co.). The particles were washed off with a stream of filtered seawater and then filtered again. The second filtration was necessary because hydrocarbons not associated with particles collected on the first filter. Hydrocarbons not associated with particles were not removed by the washing procedure. Most detrital particles and phytoplankton cells washed off the first filter, but up to 20% of the free bacterial cells could not be removed.

For radioautographic studies (G , 3H) benzo(a)pyrene (25 Ci/mM—Amersham) was added to water samples. A two-step filtration was carried out as described above, and filters from the second filtration were stained with a general bacteriological stain (3% Erythrosin B in 5% phenol solution). After several washes, slides were dipped in Kodak NTB-2 emulsion as described by Paerl and Goldman (5). After exposure and development, filters were examined with phase optics at 1200 \times to determine which particles contained radioactivity.

Analysis. Water samples were extracted with hexane (J. T. Baker—spectral grade), and an ultraviolet spectrophotometric method (6) was used to quantitate naphthalene, methylnaphthalenes, and dimethylnaphthalenes. Earlier experiments in these enclosures showed that determination of naphthalenes by gas-liquid chromatography agreed with results obtained by the ultraviolet method (3). The sensitivity of the ultraviolet method was 0.5 $\mu g/L$ for each of the various naphthalenes. To determine polycyclic aromatic hydrocarbons, other than naphthalenes, hexane extracts were concentrated to dryness under nitrogen and dissolved in 10 μL of methanol. Anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene were measured by passing 5 μL of the methanol solution through an assembled liquid chromatograph equipped with a fluorometric detector (Fluoromonitor—American Instrument Co.). The sensitivity of the method was 0.01 $\mu g/L$ for anthracene, fluoranthene, and benzo(a)pyrene and 0.02 $\mu g/L$ for benz(a)anthracene. A standard curve was prepared for each compound. The chromatograph was equipped with a LiChrosorb RP-2 (Merck) column (25 cm long and 2.0 mm i.d.). The sample was eluted with three solvent mixtures added sequentially (solvent 1—methanol:water, 1:1; solvent 2—methanol:water, 2:3; solvent 3—methanol:water, 3:2).

The concentration of total hydrocarbons in the water 1 day after the addition of oil was obtained using an infrared absorption procedure (8). One-liter samples were acidified with 1 mL of sulfuric acid (50%) and extracted with 20 mL of carbon tetrachloride (Burdick and Jackson, analytical grade). Absorbance at 2930 cm^{-1} was compared to a calibration curve constructed for solutions of the Prudhoe crude oil in carbon tetrachloride.

The procedure used to analyze volatile aromatic hydrocarbons in seawater using helium partitioning gas chromatography has been described (9). Data obtained from the gas chromatograms were treated according to the method of McAuliffe (10) to calculate aromatic concentrations.

Animal tissues were homogenized in a blender for 2 min. Sediment samples were mixed with an equal volume of water. One milliliter of tissue or sediment slurry was saponified with 0.5 mL of 4 N NaOH by heating at 95 $^{\circ}C$ for 2 h. The sample was mixed with 5 mL of hexane. The hexane extracts were analyzed by either the ultraviolet spectrophotometric method (for naphthalenes) or liquid chromatography (anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene). Between 90 and 95% of the selected aromatics was recovered when sediment and animal tissues were spiked with these compounds.

For the radiolabeled benzo(a)pyrene experiment, water was collected from the following depth intervals: 0–5, 5–10, and 10–13 m. Four liters of water were passed through a glass fiber filter. The filter was added to a liquid scintillation cocktail (Aquasol—New England Nuclear) and counted (Beckman Model LS 100C liquid scintillation counter). The values obtained were assumed to be total counts in the water. Separate extractions of the water with benzene indicated that glass fiber filters removed 70–90% of the radioactivity in the water. Benzene extracted benzo(a)pyrene, hydroxy derivatives, and quinones when these were added to water samples. In addition

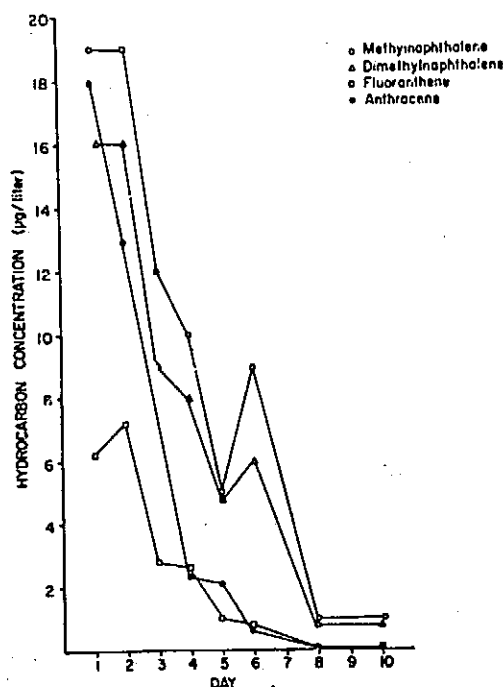


Figure 1. Polycyclic aromatic hydrocarbons from 3-m water

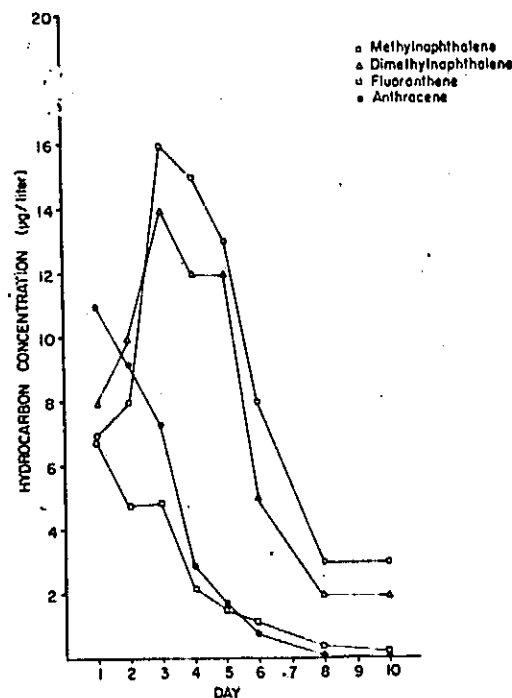


Figure 2. Polycyclic aromatic hydrocarbons from 7-m water

to determining total radioactivity, filters were also extracted with methanol:benzene (1:2 v/v). This extract was concentrated to a small volume under nitrogen in the dark, added to a silica gel thin-layer plate (Merck Co.), and run in a solvent system (ethanol:benzene, 1:9 v/v) to separate degradation products from the benzo(a)pyrene. Benzo(a)pyrene and 3-hydroxybenzo(a)pyrene and benzo(a)pyrene-1,6-dione were used as standards. The hydroxy benzopyrene and quinone were provided by A. R. Patel through the National Cancer Institute Carcinogenesis Research Program.

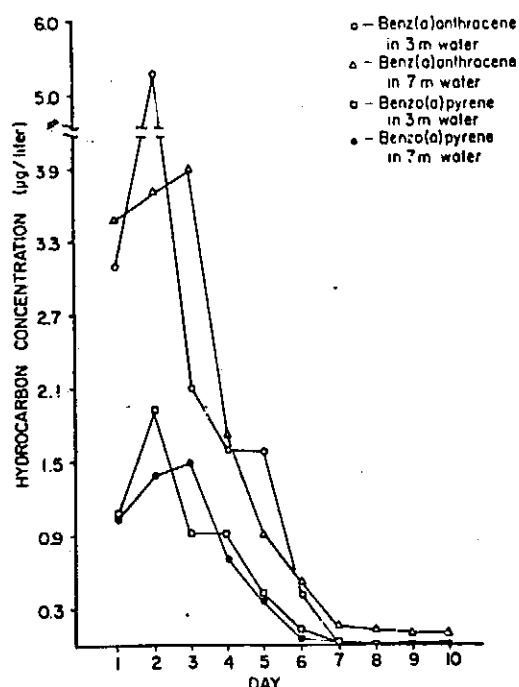


Figure 3. Benz(a)anthracene and benzo(a)pyrene from 3- and 7-m water

Results

The addition of a dispersion of 100 g of Prudhoe crude oil to the top 10 m of the water column resulted in small oil slicks on the surface which gradually disappeared over 10 days. After 1 day the nonvolatile hydrocarbon concentration at 7 m by the infrared method was 280 µg/L. The concentrations of volatile aromatic hydrocarbons, namely, benzene, toluene, ethylbenzene, xylene, and trimethylbenzenes, were 1700, 1500, 300, 540, and 1500 µg/L, respectively. No other measurements were made of total nonvolatile or volatile hydrocarbon concentrations. In similar experiments with fuel oil, volatile hydrocarbons were not detected after 3 days (11).

The concentrations of naphthalene, methylnaphthalenes, dimethylnaphthalenes, anthracene, fluoranthene, benz(a)-anthracene, and benzo(a)pyrene on day 1 were 11, 19, 16, 18, 6.2, 3.1, and 1.1 µg/L, respectively. The concentration of each hydrocarbon decreased at an exponential rate during the following 17 days of the experiment (Figures 1-4, Table I). Naphthalenes increased slightly in surface waters on day 6 (Figure 1), which may be due to more of these components dissolving in the water from the surface slick. A liquid chromatogram of a day 3 water sample from 7 m is shown in Figure 5 where the concentrations of anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene were 7.3, 4.9, 3.9, and 1.5 µg/L, respectively. Use of the high-pressure liquid chromatograph with a fluorescence detector allowed separation and detection of these polycyclic aromatic hydrocarbons in water, sediment, zooplankton, and oysters during the course of the experiment. The time for the hydrocarbons to decrease to 50% of their original values, i.e., half-life, was 3-4 days in water from 3 m and 4-6 days in water from 7 m. By day 17 all of the spiked hydrocarbons were below the level of detection in water from 3 m.

A phytoplankton bloom, predominantly centric diatom species, with an initial chlorophyll-a concentration of 19 µg-atoms/L, and a phytoplankton carbon concentration of 3 mg/L was captured in the enclosure. This bloom rapidly depleted nutrients, and the phytoplankton sank to the bottom

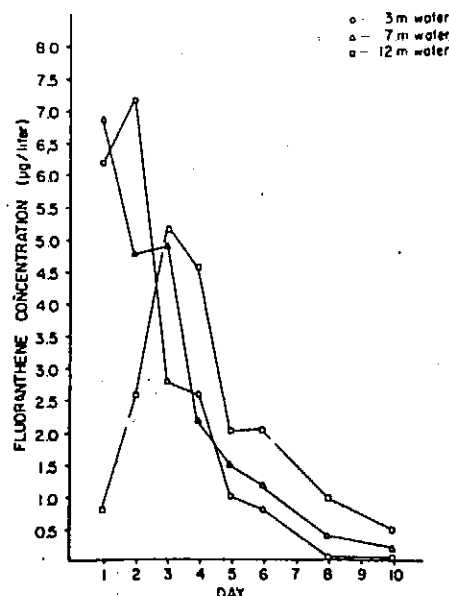


Figure 4. Changes in fluoranthene concentration with depth and time.

Table I. Addition of ³H-Benzo(a)pyrene to an Enclosure—Concentration Changes of Hydrocarbon and Degradation Products

Day	Sample depth (m)	Total benzo(a)pyrene and degradation products (µg/L)	Benzo(a)pyrene (µg/L)	Hydroxylated derivatives and quinones (µg/L)
1	0-5	1.4	1.2	0.08
1	5-10	1.3	1.1	0.05
1	10-13	0.1	0.1	0
2	0-5	1.1	0.7	0.01
2	5-10	0.9	0.6	0.01
2	10-13	0.2	0.2	0
3	0-5	0.9	0.5	0.1
3	5-10	0.9	0.6	0.1
3	10-13	0.4	0.2	0.02
4	0-5	0.9	0.3	0.2
4	5-10	0.7	0.5	0.1
4	10-13	0.6	0.4	0.01
6	0-5	0.4	0.2	0.1
6	5-10	0.3	0.2	0.1
6	10-13	0.3	0.2	0.02
8	0-5	0.2	0	0.07
8	5-10	0.6	0.4	0.05
8	10-13	0.4	0.3	0.04
10	0-5	0.1	0	0.02
10	5-10	0.2	0.1	0
10	10-13	0.2	0.2	0

of the enclosures, which resulted in a large decrease in chlorophyll-a in the water of both control and treated enclosures. Similar cycles of centric diatom blooms, depletion of nutrients, and subsequent sinking of the phytoplankton are a normal summer feature of Saanich Inlet (12). Hydrocarbons associated with the phytoplankton are carried to the bottom sediments by this sinking process. Analysis of fluoranthene in water from 3, 7, and 12 m showed the path of fluoranthene through the water column. On day 1 fluoranthene was 6.2 µg/L at 3 m but decreased to 2.8 µg/L by day 3 with subsequent increase in the water from 7 and 12 m and eventual incorporation into the sediment (Figure 4 and Table II).

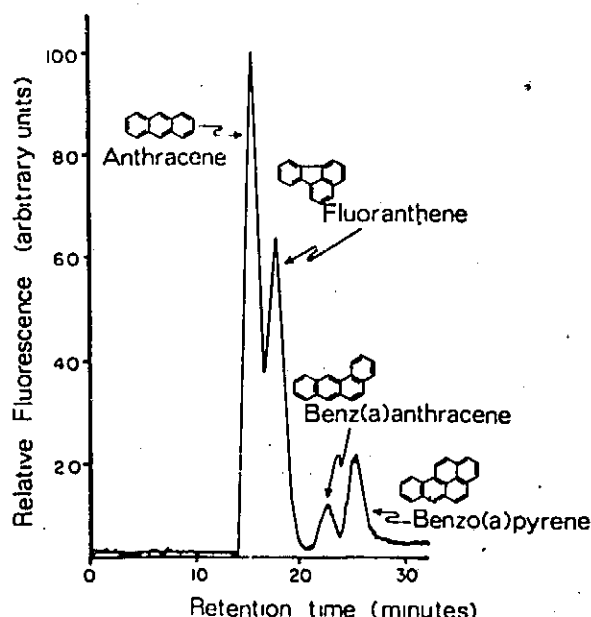


Figure 5. Liquid chromatogram of 7-m water on day 3

Photochemical Oxidation. The importance of photochemical oxidation can only be inferred from our results. The higher weight polycyclic aromatic hydrocarbons, in particular benzo(a)pyrene and benz(a)anthracene, are susceptible to photooxidation (13). The short wavelengths required for photooxidation of these compounds penetrate only a few meters in the ocean, and should be even more attenuated in the presence of a phytoplankton bloom. The experiment with tritiated benzo(a)pyrene suggests that photooxidation of this compound was important in the top 5 m (Table I). On day 4, 30% of the radioactivity collected in the upper 5 m of the enclosure was due to benzo(a)pyrene. On thin layer plates the remaining radioactivity was collected in the area of quinones and more polar compounds. Benzo(a)pyrene accounted for the majority of the radioactivity in bottom waters and sediments. Approximately 40% of the benzo(a)pyrene in the waters of the oil-treated enclosures was recovered in the sediment, suggesting that no more than 50% of the benzo(a)pyrene could have been photooxidized (Table III).

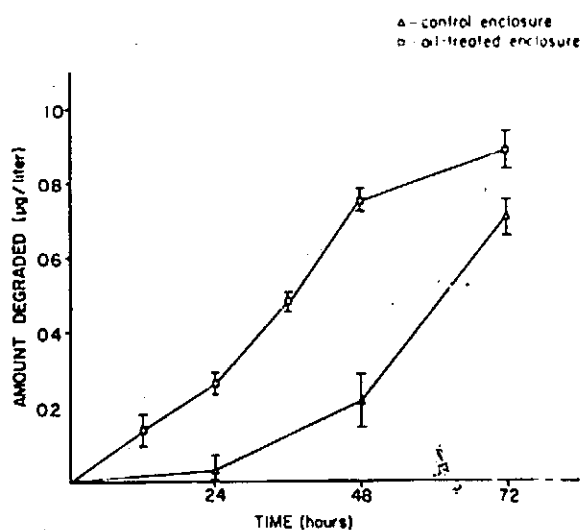


Figure 6. Microbial degradation of ^{14}C -1-methylnaphthalene (25 $\mu\text{g/L}$) in oil-treated and control enclosures

Microbial Degradation. The rate of microbial degradation was evaluated by the addition of ^{14}C -labeled hydrocarbons to water samples from the enclosures and later measurement of $^{14}\text{CO}_2$ produced. Radiolabeled benz(a)anthracene and benzo(a)pyrene were not degraded in water in the dark from either the treated or control enclosures. Anthracene was not degraded in the control water but slowly degraded (0.02 $\mu\text{g/L/day}$) in water from the treated enclosure. No degradation was observed in mercury treated controls. As a result of adding oil to an enclosure, radiolabeled naphthalene and methylnaphthalene were degraded on day 3 at a rate of approximately 0.4 $\mu\text{g/L/day}$ (Figure 6; only the degradation of methylnaphthalene is shown, but very similar curves were obtained for naphthalene). If the amount of unlabeled methylnaphthalene in the water (12 $\mu\text{g/L}$) is taken into account, the calculated degradation rate is 0.5 $\mu\text{g/L/day}$. Assuming a uniform rate throughout the enclosure, then 30 mg of methylnaphthalene would be degraded to CO_2 in one day. On day 3 there was approximately 600 mg of methylnaphthalene in the water of the oil-treated enclosure. Thus up to 5% of the naphthalenes in the water could be degraded daily. Water in the control enclosure showed an initial lag in the degradation of naphthalene

Table II. Aromatic Hydrocarbons in Bottom Sediments from Oil-Treated Enclosure

Time after oil addition (days)	Naphthalene		Methylnaphthalenes		Dimethylnaphthalenes	
	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)
2	70	28	71	28	169	68
4	15	9	22	13	68	40
7	5	3	5	3	22	12
10	7	6	10	9	30	26
14	2	2	3	3	6	7
17	<0.5	<1	<0.5	<1	<0.5	<1

Time after oil addition (days)	Anthracene		Fluoranthene		Benz(a)anthracene		Benzo(a)pyrene	
	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)	Concn ($\mu\text{g/g}$)	Amt in sediment (mg)
2	<0.2	<0.1	<0.2	<0.1	<0.4	<0.2	<0.2	<0.1
4	1.8	1.1	2.4	1.4	2.2	1.3	0.8	0.5
7	5.3	2.9	4.4	2.4	5.4	3.0	2.1	1.2
10	3.5	3.1	2.8	2.5	3.8	3.3	1.3	1.1
14	5.1	5.6	6.2	6.8	10.0	11.0	4.9	5.4
17	0.4	11.8	10.0	14.0	24.0	33.6	6.0	8.4

Table III. Total Amount of Aromatic Hydrocarbons in Bottom Sediments and Water

Hydrocarbon	Estimated amt in water on day 1 (mg)	Total in sediment (mg)	% recovered in sediment
Naphthalene	440	48	1
Methylnaphthalenes	780	58	7
Dimethylnaphthalenes	480	153	32
Anthracene	600	25	4
Fluoranthene	278	27	10
Benz(a)anthracene	132	52	39
Benzo(a)pyrene	44	17	39

and methylnaphthalene, but after incubation with the labeled hydrocarbons for 72 h the amount degraded was nearly the same as in the oil treated enclosure (Figure 6).

Sedimentation and Adsorption Studies. Determination of the concentration and amount of the hydrocarbons in the bottom sediments at various times after addition of oil allowed us to evaluate the importance of sedimentation processes in removing hydrocarbons from the water. Most of the naphthalenes, with a predominance of dimethylnaphthalenes, appeared in early sediment samples, i.e., during the first 7 days, whereas the less soluble higher weight aromatics appeared in later samples. A comparison between the amount of hydrocarbon in the water column on day 1 (estimated by assuming all of the hydrocarbon was in the top ten meters) and the total collected in the sediment is shown in Table III. The percent recovered in the sediment varied from 4% for anthracene to 39% for benzo(a)pyrene.

The hydrocarbons in the bottom sediments were presumably carried there by particles originally in the water column. Particles that could take up hydrocarbon included phytoplankton, detritus, and zooplankton. Zooplankton were sampled on days 4 and 7. On day 4 the concentrations of anthracene, fluoranthene, benzo(a)pyrene, and benz(a)anthracene in a zooplankton sample, predominantly the copepod *Pseudocalanus minutus*, were 3.3, 1.4, 0.3, and 1.2 µg/g, respectively. These concentrations were less than those in the bottom sediment after day 4 (Table II). After 9 days the concentration of these compounds in zooplankton was below detectable levels, i.e., less than 0.1 µg/g. The ability of copepods and other zooplankton to degrade and discharge hydrocarbons may account for this decrease (14-16).

On day 5 a water sample from 12 m was analyzed before and

after filtering through a glass fiber filter. Less than 10% of the benzo(a)anthracene and benzo(a)pyrene and 50% of the anthracene and fluoranthene passed through the filter. For laboratory adsorption studies, radiolabeled hydrocarbons were added to water samples collected from the control enclosure. After a 2 h incubation period, 2% of the radiolabeled naphthalene and methylnaphthalene at a concentration of 25 µg/L were taken up by suspended particles. The relative adsorption of anthracene (15 µg/L), benz(a)anthracene (3 µg/L), and benzo(a)pyrene (2 µg/L) to suspended particles were 11, 59, and 75%, respectively. Radioautographs with tritiated benzo(a)pyrene added to water samples indicated that this hydrocarbon was associated with detrital particles rather than with living phytoplankton cells. Under the microscope these detrital particles appeared to be aggregates of dead phytoplankton cells and bacteria.

Hydrocarbon Uptake by Oysters. Oysters suspended in the oil treated enclosure rapidly took up all hydrocarbons with very high accumulation of naphthalenes (Table IV). For depuration experiments, oysters were transferred to water outside the enclosure. Naphthalenes were rapidly released and were not detectable after 23 days. There was a much slower release of accumulated anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene. They were still present at the end of the 23 days. Based on these depuration experiments, calculated half lives of the naphthalenes, assuming exponential discharge, were 2 days; whereas for anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene, the half-lives were 3, 5, 9, and 18 days, respectively. Thus the higher weight aromatics appear to persist for longer periods than the lower weight naphthalenes. Other depuration studies with mussels collected from oil contaminated waters have indicated the persistence of higher weight aromatics (17, 18).

Discussion

We propose that the primary processes effecting the concentration of the hydrocarbons in the waters of the enclosures were evaporation, microbial degradation, sedimentation, and photochemical oxidation. Based on the data presented here and from other reported studies, we have attempted to evaluate the relative importance of these processes as they effect the concentration of the different aromatics.

Most studies on evaporation have been concerned with hydrocarbon losses from an oil slick. Hydrocarbons below C₁₅ (BP -270 °C) volatilize in a few days from an oil slick (19-21). In our experiments loss of naphthalenes from the upper waters, particularly naphthalene (BP -210 °C) would be pre-

Table IV. Uptake and Depuration of Aromatic Hydrocarbons by Oysters from Oil-Treated Enclosure

Time of exposure (days)	Depuration time (days)	Naphthalene		Methylnaphthalenes		Dimethylnaphthalenes			
		Oyster (µg/g)	Water (µg/L)	Oyster (µg/g)	Water (µg/L)	Oyster (µg/g)	Water (µg/L)		
2		30	5	58	8	84	10		
8		12	3	36	3	72	2		
2	7	1	...	1	...	8	...		
8	7	2	...	2	...	4	...		
8	23	nd ^a	...	nd	...	nd	...		
		Anthracene		Fluoranthene		Benz(a)anthracene		Benzo(a)pyrene	
		Oyster (µg/g)	Water (µg/L)	Oyster (µg/g)	Water (µg/L)	Oyster (µg/g)	Water (µg/L)	Oyster (µg/g)	Water (µg/L)
2		5.6	13	5.0	7.2	2.8	5.3	0.36	1.9
8		2.5	1	4.0	0.4	1.8	0.1	0.30	0.1
2	7	1.2	...	1.7	...	1.9	...	0.40	...
8	7	0.4	...	1.4	...	1.0	...	0.20	...
8	23	0.1	...	0.4	...	0.3	...	0.12	...

^a nd, not detectable, less than 0.5 µg/g.

dicted. Hydrocarbons in the C_{15} - C_{25} range (BP 250-400 °C), anthracene and fluoranthene in our experiments, are volatilized from an oil slick only to a limited extent.

Microbial degradation was measurable only for naphthalenes and anthracene in the oil treated enclosure. Water from the control enclosure required incubation with ^{14}C naphthalenes for 48 h before appreciable degradation occurred, whereas in water from the oil treated enclosure, measurable degradation of ^{14}C naphthalenes took place within 4 h after addition. An earlier hydrocarbon experiment showed marked increases in microbial degradation rates of naphthalenes 3 days after hydrocarbon addition (7). Several studies have shown higher numbers of oil degrading microbes in oil polluted areas relative to nearby "clean" areas (22, 23).

Photochemical oxidation, involving free radical addition catalyzed by light or metal ions, appears to be an important process in the destruction of oil slicks (21). Based on laboratory studies it appears that anthracene, fluoranthene, benz(a)anthracene, and benzo(a)pyrene of the aromatics studied in our experiments are subject to photochemical oxidation in slicks or near surface waters (13, 24, 25). Benzo(a)pyrene is the most susceptible of these compounds to photooxidation, and the results of the experiment involving addition of tritiated benzo(a)pyrene to an enclosure suggested that photochemical oxidation was important in the surface waters. Approximately 40% of the benzo(a)pyrene in the water was recovered in the bottom sediments. Since microbial degradation and evaporation were not important, it is suggested that as much as 50% of the benzo(a)pyrene was photooxidized during the 17 days of the experiment. It is probable that there were losses to the side of the plastic enclosure and incomplete recovery of hydrocarbon in the sediment so that photooxidation was less than 50%.

Sedimentation is important in carrying hydrocarbons from both oil slicks and from the water to the bottom (21). Particles such as fine grained clays and detritus can adsorb dissolved, dispersed, or particulate hydrocarbons (26-29). Experimental oil spills have shown rapid hydrocarbon decreases in the water after disappearance of the oil slick with increases of the aromatics in the sediments (29, 30).

In our experiments, naphthalenes, particularly dimethylnaphthalenes, were found in the bottom sediments during the first 7 days, whereas the heavier weight aromatics were slower to sediment. This suggests that the mechanisms for carrying naphthalenes to the bottom were different than those of the other aromatics. Soto et al. (31) showed active uptake of naphthalene by phytoplankton. We suggest that naphthalenes, but not the larger aromatic hydrocarbons, were actively taken up by living phytoplankton cells which subsequently sank to the bottom. Radioautographs with tritiated benzo(a)pyrene indicated that this hydrocarbon, and presumably other higher weight aromatics, were associated with detritus, composed of clumps of dead phytoplankton cells and associated bacteria. These particles probably sank at a slower rate than living phytoplankton cells. Figure 3 shows a large increase in naphthalenes in 7 m water on days 3 and 4, which was not observed with the other aromatics. Presumably this increase was due to naphthalenes in surface water phytoplankton being carried to deeper waters. The lower concentration of naphthalenes in later sediment samples (after 7 days) could be due to increases in the microbial degradation rates of these compounds in sediment or bottom waters. Since it appears that phytoplankton take up but cannot metabolize naphthalenes (31), autolysis of the phytoplankton cells may release naphthalenes that are degraded.

It is difficult to explain the loss of anthracene from the enclosure. The rate of decrease in the water was very rapid (Figures 1 and 2) with only small increases seen in the 7- and 12 m water samples between days 2 and 4. Other aromatics

increased on these days as particles with associated hydrocarbons were carried to the bottom. Only 1% of the anthracene in the water was recovered in the bottom sediments. The microbial degradation rate was very low for anthracene. With a boiling point of 340 °C, evaporation was not expected to be important, although laboratory studies have shown up to 15% loss of anthracene from solution (32). Possibly, photochemical oxidation was extremely rapid for this compound.

Conclusions

Changes in the concentration of various fluorescent aromatics in water, sediment, zooplankton, and oysters from an oil treated enclosure were easily determined by using crude oil enriched with the compounds of interest and a liquid chromatograph with a fluorescence detector. The sensitivity of the method allowed detection of 0.01 $\mu g/l$ of the individual hydrocarbons.

Our results indicated that aromatic hydrocarbons have short residence times, i.e., on the order of a few days, in marine waters. For lower molecular weight aromatics, such as benzenes, naphthalenes, anthracenes, and phenanthrenes, microbial degradation and evaporation are the primary removal processes. The concentrations of higher weight aromatics, such as chrysenes, benzanthracenes, and benzpyrenes, are primarily effected by sedimentation and photochemical oxidation. Because of their low solubility in water, the higher weight aromatics are associated with particles in the water. After sedimentation of higher weight aromatics, biological degradation by interactions between macrofauna, meiofauna, and microfauna of the sediment becomes an important factor in their removal (23, 33). In studies now being conducted we are adding bottom material collected from an oil-treated enclosure to trays of marine sediments to determine rates of hydrocarbon degradation in the sediment. When oil from a spill is incorporated into fine sediments, it may persist for many years with storms and tides sometimes causing resuspension of these sediments with their associated hydrocarbons (34).

In open ocean areas with low concentrations of suspended particles, rates of hydrocarbon sedimentation would be low, but because of the clear waters, photochemical degradation rates should be high. In the case of an oil spill, hydrocarbons should continue to be found in the water as long as a slick persists followed by rapid decreases in their concentration as a result of the various natural removal processes.

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Received for review October 31, 1977. Accepted January 25, 1978.
Work supported by National Science Foundation/DOE Grants
GX-39149 and OCE76-84108.

Rapid Weathering Processes of Fuel Oil in Natural Waters: Analyses and Interpretations

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■ The fate-determining steps of weathering petroleum in the aquatic environment were studied in model experiments. Capillary column gas chromatography and infrared absorption measurements showed different weathering processes for No. 2 fuel oil, depending on the turbulence and the level of suspended solids (kaolinite) in water during experiments. Partial dissolution, adsorption, dispersion, and agglomeration of No. 2 fuel oil initially occurred and resulted in the fractionation of the original oil mixture. Alkylated benzenes and naphthalenes were enriched in the water phase (up to 5 mg/l.), certain aliphatic hydrocarbons above mol wt 250 were adsorbed onto kaolinite (200 mg/kg), and oil droplets were agglomerated with suspended minerals (20 g/kg) after increased turbulence. The same fractionation pattern was observed for a ground water oil spill, although the oil was already biochemically altered.

One would like to be able to characterize the processes that influence the fate of oil in the aquatic environment. A number of authors (1) have described various physical and biological processes that contribute to the distribution and degradation of petroleum products in the aquatic environment. Difficulties have arisen in determining the relative magnitude of the various processes. Reliable information on the distribution of oil components in aquatic environments is important because the more water soluble aromatic hydrocarbons, even in minute concentrations, may have toxic effects on aquatic organisms (2).

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This work presents an analysis of the initial short-term processes affecting the distribution of fuel oil in surface water containing a suspended clay mineral. Processes studied include dissolution, suspension, agglomeration, and adsorption. Evaporation was not studied, since surface oil spills generally contribute less to oil pollution than continuous inputs from diffuse sources (3).

Model experiments were done with No. 2 fuel oil and water containing suspended particulates. Then a comparison was made of these model experiments and an actual case involving polluted ground water.

Experimental

Model Experiments. A 250-mL erlenmeyer flask was filled with distilled water containing sodium chloride (10^{-3} M) and sodium bicarbonate to stabilize the pH at 7.5. To withdraw samples, a tube was placed in the flask, with one end near the bottom and the other end outside. Ten milliliters of No. 2 fuel oil were carefully placed on top of the water body with a pipet, avoiding single oil droplets. Flasks were then stirred with a magnetic stirrer at a constant rate (1/s). Different flow conditions were achieved by changing the length of the magnetic stirrer: 2 cm for low intensity, 10 cm for high intensity (oil/water interface disrupted). A specific surface (10 m²/g) of kaolinite (0.2 μ m) with a relatively low surface area (10 m²/g) were used as the particulate material. Experiments were performed for a period of 24 h at 20 °C as follows: I: oil/water without suspended solids, low stirring intensity; II: oil/water with suspended solids, low stirring intensity; III: oil/water with suspended solids, high stirring intensity; IV: gasoline/water without suspended solids, low stirring intensity.

DATA EVALUATION RECORD

TD15-0030

DATA EVALUATION RECORD

PAGE 1 OF

CASE G50022

NAPHTHALENE

STUDY 66

PM 400 11/20/79

CHEM 055001

Naphthalene

BRANCH EFR

DISC 20 TOPIC 1005

GUIDELINE 40 CFR 163.62-843

FORMULATION NA - ACTIVE INGREDIENT

FICHE/MASTER ID 05006779

CONTENT CAT 01

Lee, R.F.; Gardner, W.S.; Anderson, J.W.; Blaylock, J.W.;
Harwell-Clarke, J. (1978) Fate of polycyclic aromatic
hydrocarbons in controlled ecosystem enclosures. Environmental
Science and Technology 12(7):832-838.

SUBST. CLASS = S.

OTHER SUBJECT DESCRIPTORS

PRIM: EFR -30-052020

EEB -35-10000047

DIRECT RVW TIME = 9

(MH) START-DATE

END DATE

REVIEWED BY: D. Harper and R. Hebert

TITLE: Staff Scientists

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SIGNATURE:

Daniel Harper, Richard R Hebert

DATE: Nov. 14, 1980

APPROVED BY:

TITLE:

ORG:

LOC/TEL:

SIGNATURE:

DATE:

CONCLUSIONS:

Metabolism - Effects of Microbes on Pesticides

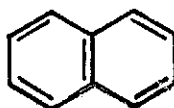
1. This portion of the study is scientifically valid.
2. [1-¹⁴C]Naphthalene was degraded in seawater by unidentified microorganisms at 0.4 µg/liter/day during a 3-day test period.

Accumulation - Fish

1. This portion of the study is scientifically valid.
2. Naphthalene accumulates in the oyster Crassostrea virginica and is rapidly depurated. An accumulation factor of 6,000 was observed on the second day of exposure to an initial naphthalene concentration of 418 ppb in a spiked oil-treated model ecosystem. The maximum accumulation factor cannot be determined from the data. Depuration from oysters containing 12-30 ppm was rapid, with only 1-2 ppm remaining after 1 week.

MATERIALS AND METHODS:

NAPHTHALENE, ALBOCARBON, CAMPHOR TAR,
DEZODORATOR, MOTH BALLS, MOTH FLAKES,
NAPHTHALIN, NAPHTHALINE, NAPHTHENE,
TAR CAMPHOR, WHITE TAR



Naphthalene

One gram of naphthalene (Aldrich Chemical Co.; purity unspecified) and varying amounts of six other aromatic hydrocarbons were added to a mixture of 100 g of Prudhoe crude oil (containing an estimated 80 mg of naphthalene), 1 liter of acetone, 0.5 liters of ethanol, and 40 liters of seawater. The mixture was stirred, and the dispersion was pumped into a polyethylene-enclosed cylinder (2-m diameter and 15 m deep) containing 60,000 liters of seawater. One liter of acetone and 0.5 liters of ethanol were added to a control enclosure. A cage of oysters (*Crassostrea virginica*) was suspended at 7 m in the oil-treated enclosure. The enclosures were kept at 12 C.

At various time intervals, water samples were collected from three depths by using either a peristaltic pump or a Niskin sampler. Samples were extracted with hexane, and a UV spectrophotometric method was used to quantitate naphthalene. The level of sensitivity was 0.5 µg/liter, as verified by gas-liquid chromatography, and recovery levels were identical for each sampling method.

Oyster samples were obtained after 2 and 8 days of exposure and after 7 and 23 days of depuration (cage transferred to fresh seawater). The samples were homogenized in a blender for 2 minutes. Samples of bottom sediment were collected by pumping the sediment through pipes connected to the bottom of the enclosure. One milliliter of tissue or sediment was saponified with 0.5 ml of 4 N NaOH by heating at 95 C for 2 hours. The sample was mixed with 5 ml of hexane, and the hexane extracts were analyzed by UV spectrophotometry. Naphthalene recovery levels were 90-95%.

Radiolabeled hydrocarbons, including [1-¹⁴C]naphthalene (Amersham), were separately dissolved in 2 µl of acetone and added to 100-ml water samples collected at an unspecified time from 3- and 7-m depths. The final naphthalene concentration was 25 µg/liter. The flasks were sealed and incubated at 12 C in the dark for various time intervals, and the respired ¹⁴CO₂ released after the addition of acid (unspecified method) was collected on filters soaked with phenethylamine and counted by liquid scintillation counting (LSC). Control flasks were sterilized with 5 mg HgCl₂.

Radiolabeled naphthalene at 25 $\mu\text{g/liter}$ was also added to 100-ml water samples and incubated at 12 C for 3 hours to allow for adsorption to suspended particles. The water was filtered onto a 0.4- μm filter, washed with filtered seawater, and filtered again. Most detrital particles and phytoplankton were washed off, but up to 20% of the bacteria were not removed. Filters were counted by LSC.

REPORTED RESULTS:

The concentration of naphthalene on day 1 was 11 $\mu\text{g/liter}$. It decreased exponentially during the 17-day experiment. The half-life was 3-4 days in water from 3 and 4-6 days in water from 7 m. By day 17 naphthalene levels were below the limit of detection in water from 3 m. Naphthalene was present in bottom sediment samples at 70 $\mu\text{g/g}$ on day 2 and at <0.5 $\mu\text{g/g}$ on day 17.

Naphthalene was accumulated by oysters; 30 and 12 $\mu\text{g/g}$ had accumulated on days 2 and 8, respectively. Naphthalene was rapidly depurated by oysters (Table 1).

[^{14}C]Naphthalene was degraded by microorganisms at a rate of 0.4 $\mu\text{g/liter/day}$ during the first 3 days of incubation of the 100-ml samples. After 3 hours, 2% of the radiolabeled naphthalene was adsorbed by suspended particles.

DISCUSSION:

1. Neither the maximum concentration of naphthalene accumulated by the oysters nor the depuration half-life can be determined from the data presented.
2. Zooplankton samples were also removed for analysis, but data are not reported for naphthalene.
3. Specific data for microbial metabolism were not given.
4. The initial concentration of naphthalene was not determined. The amount of naphthalene in Prudhoe oil has never been determined. Kuwait oil is known to contain ~80 mg naphthalene/100 g oil. As Kuwait and Prudhoe oils are similar, it was assumed that 100 g of Prudhoe oil also contains ~80 mg naphthalene. In this study, the 100 g of Prudhoe oil added to the enclosure was first supplemented with 1 g naphthalene. Therefore the initial naphthalene concentration can be estimated as 1,080 mg/60,000 liters, or ~18 ppb.